

The University of Chicago

HISTOLOGICAL AND
REGENERATIVE STUDIES ON THE
FLAX SEEDLING

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE BIO-
LOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BOTANY

1933

By

DONALD MUNDELL CROOKS

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CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 446

DONALD M. CROOKS

(WITH FORTY-FOUR FIGURES)

Introduction

Common flax, *Linum usitatissimum* L., is the only member of the Linaceae of economic importance (6, 11). The fibers are a source of linen, the seed is a commercial source of linseed oil, and the residue after the oil is pressed from the seeds is an important source of food for livestock. Most of the early studies of the plant were made in Europe (4, 5, 6, 7, 8, 15, 16, 17, 18). Critical investigations of all phases of fiber development and of the commercial use of flax have been carried on in Holland, Germany, Russia, and in the United States by the Department of Agriculture.

Histological studies on the seedling were confined almost entirely to Europe during the last quarter of the 19th century. GÉRARD (5) and TOGNINI (15) reported investigations of the transition from root to stem; JANCZEWSKI (7) classified the histogens of the primary root; VAN TIEGHEM (17) described the origin of the lateral roots; and WILDE (18) described transverse sections of the mature root, stem, and leaf. TSCHIRCH and OESTERLE (16) described the fruit, reported large aleurone bodies and no starch in the cotyledons, and gave a brief description of germination. In most of these studies flax was selected merely for morphological comparison as a representative of the Linaceae. Certain phases of vegetative regeneration of shoots were observed by REICHARDT (12). His observations were verified by TAMMES (13) in Holland, and the histology of the regenerated parts was investigated by BEALS (3) in the United States. HERZOG (6) deals with the botanical, cultural, and commercial aspects of the plant. Extensive bibliographical lists are given by HERZOG (6) and TAMMES (13). The literature citations included in this paper are

limited to those directly concerned with some phase of seedling histology. The present paper supplements the studies previously made on the histology, anatomy, and regeneration of flax seedlings, and deals in particular with the ontogeny of tissues and organs.

The Bison variety of flax, which is widely grown in North and South Dakota, was used in this investigation. Plants were grown in the greenhouse at the University of Chicago during the winter of 1931-32 and compared with material grown in gardens during the following summer at Ball State Teachers College, Muncie, Indiana. Material from these sources showed no morphological differences except a slightly longer axis in some of the greenhouse plants.

Various fixatives were used. The most satisfactory results were secured with a modification of Nawaschin's solution which consists of solution A (7 cc. glacial acetic acid, 1 gm. chromic acid crystals, and 92 cc. distilled water) and solution B (30 cc. formalin and 70 cc. water) mixed in equal volumes just before using. A solution made of 5 cc. of formalin, 5 cc. of glacial acetic acid, and 90 cc. of 50 per cent alcohol gave fair results except when it was desired to secure mitotic figures and a minimum shrinkage of the protoplasm. The greatest number of division figures were obtained in roots killed at 11 P.M. and in stem tips and leaves killed at 1 P.M. Chloroform was used for clearing. Final infiltration of paraffin was accomplished very slowly, in two stages: (1) by evaporating the chloroform from the paraffin-chloroform mixture in an evaporating dish placed on top of the oven for a period of about 48 hours; and (2) by transferring to an oven at 55° C. until no taste of chloroform remained.

Serial sections were cut 6-15 μ in thickness and stained in a modification of Flemming's triple stain. All drawings (except figure 3) were made by the use of a microprojection apparatus.

Germination and young seedling

Under greenhouse conditions seeds germinated rapidly at a temperature of 65°-90° F. At a temperature of 65°-70° F. the cotyledons showed above the ground in four or five days, and the seedlings appeared much the same as summer-grown plants. Higher temperatures gave more rapid germination, but after a few days the hypocotyl of the seedlings elongated greatly, as compared with those

grown outside during the summer or in cooler temperatures in the greenhouse.

In the process of germination the primary root emerges from the seed coat by the end of the first or second day and grows downward, thus forming a type of tap root which becomes the axis of the entire root system (2). Before the cotyledons show above the surface of the soil the hypocotyl region, in response to elongation in the basal portion, bends to the shape of an inverted U, so that the seed coat and cotyledons are pulled above the ground (10). Sometimes the cotyledons are pulled out of the seed coat while yet in the soil. The hypocotyl straightens out in about five days and the remainder of the seed coats is pushed off by the enlarging cotyledons. After a period of general growth of the hypocotyl, the first major zone of elongation is at the ground level. This zone of elongation progresses upward along the hypocotyl to the cotyledons, ceasing at the upper level in about 15–20 days. In five or six days several lateral roots appear on the primary root and are usually formed within 3 cm. of the surface of the soil. Most of the lateral roots originate from the upper 8 cm. of the primary root.

As the cotyledons push from the seed coat, they spread apart and continue to expand for several days before the epicotyl begins to elongate. The cotyledons are obovate and almost sessile, and become true photosynthetic leaves. The epicotyl grows very slowly at first. By 12–15 days it appears as a compact bud of miniature leaves with almost no elongation of internodes. After about 20 days the elongation of internodes causes very rapid growth in height. At maturity the first internode is about 1 mm. long, the second about 15 mm., and all others are usually from 5 to 10 mm. Most of the leaves are simple and sessile, although several plants have one or two double leaves between the fifth and tenth node (fig. 25).

Primary root

The primary root is diarch, with three to five well defined protoxylem elements alternating with two rather large groups of small primary phloem cells (fig. 15). The metaxylem elements are successively larger toward the center. The pericycle, which is differentiated early, is a single layer of cells. The Casparian strips of the

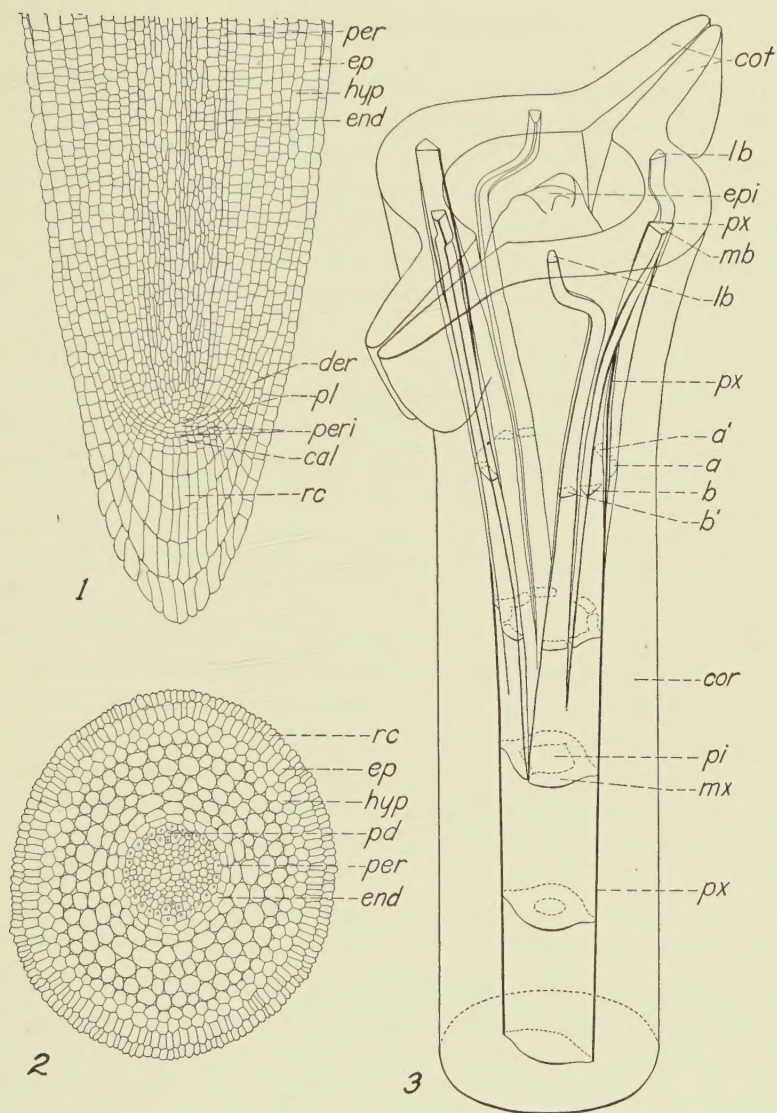
endodermis never become very thick but are deposited on the radial walls before the metaxylem is completely differentiated. The cortical cells are very regular with large intercellular spaces. The outer layer of the cortex forms a definite hypodermis which appears much as the epidermis, except that none of the cells elongate and become root hairs. The cortex persists after considerable secondary thickening, and the first breakdown of cortical cells occurs in the region midway between the endodermis and hypodermis.

Development of the root axis is by clearly defined histogens (fig. 1) which are similar to those described by JANCZEWSKI (7). The plerome and periblem give rise to the stele and cortex respectively; while a layer of cells, which makes up the calyptrogen and dermatogen overlying the periblem, produces the epidermis and the root cap.

The calyptrogen-dermatogen layer of cells overlying the periblem multiplies by periclinal divisions around the tip and forms a root cap of regular, radial rows of cells. In the lateral portion of the same layer the epidermis is formed by anticlinal divisions. Laterally, where the divisions are anticlinal the histogen is strictly a dermatogen, while the layer at the tip is a calyptrogen. The root cap at the tip is very regular because the cell divisions in the calyptrogen are periclinal only. Occasionally some of the cells in the lateral portion of the root cap divide anticlinally and distort the radial rows. This occurs only in lateral portions of the root cap and not at the tip, where the rows of cells are regular.

The cortex is derived from the periblem, which consists of two layers of cells overlying the plerome. The outermost layer of the periblem divides only anticlinally and forms the outer layer of the cortex, the hypodermis. The inner layer divides in all planes and forms the remainder of the cortex, which at maturity is about 6-9 cells in thickness (figs. 1, 2).

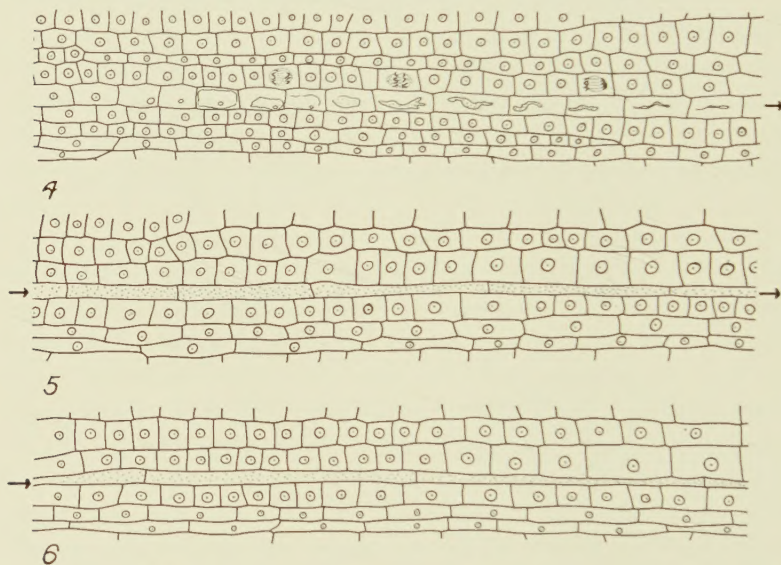
The stele is differentiated from a small group of plerome cells which divide in all planes at the tip, while divisions at a higher level are chiefly in a transverse plane. The outer layer of this group produces the pericycle, which can be distinguished rather early by the relatively dense cytoplasm and larger size of the cells. The first evidence of differentiation of the vascular system appears about 0.4 mm. above the tip of the plerome. Two primary phloem ducts are



FIGS. 1-3.—Fig. 1, median longitudinal section of primary root: *per*, pericycle; *ep*, epidermis; *hyp*, hypodermis; *end*, endodermis; *der*, dermatogen; *pl*, plerome; *peri*, periblem; *cal*, calyptrogen; *rc*, root cap. Fig. 2, transverse section 0.7 mm. from tip of primary root: *pd*, primary phloem duct.

Fig. 3, schematic diagram of vascular system of hypocotyl showing transition from root to cotyledons: *cot*, basal portion of cotyledons; *lb*, lateral bundle of cotyledon; *epi*, epicotyl; *mb*, median double bundle of cotyledon; *px*, protoxylem; *mx*, metaxylem; *pi*, pith; *cor*, cortex; *a*, *b*, bundles which anastomose and form double bundle of cotyledon; *a'*, *b'*, lateral metaxylem elements which form lateral bundles of cotyledon.

first differentiated opposite each other by the elongation and breakdown of a single row of cells lying next to the pericycle, and alternate with the two protoxylem points which are differentiated later. Figures 4, 5, and 6 show how these ducts are formed and stretched by the elongation of neighboring cells. These ducts collapse after becoming greatly stretched but usually persist until the protoxylem elements are well differentiated. Elongation of the root often causes



FIGS. 4-6.—Longitudinal sections of primary root showing formation of primary phloem duct at 0.6, 1, and 1.5 mm. from tip.

the protoxylem to collapse. The metaxylem elements differentiate centripetally until all the elements of the xylem plate are lignified and no central parenchyma remains. The outermost metaxylem elements have scalariform wall thickenings while those centrally located have pitted wall thickenings.

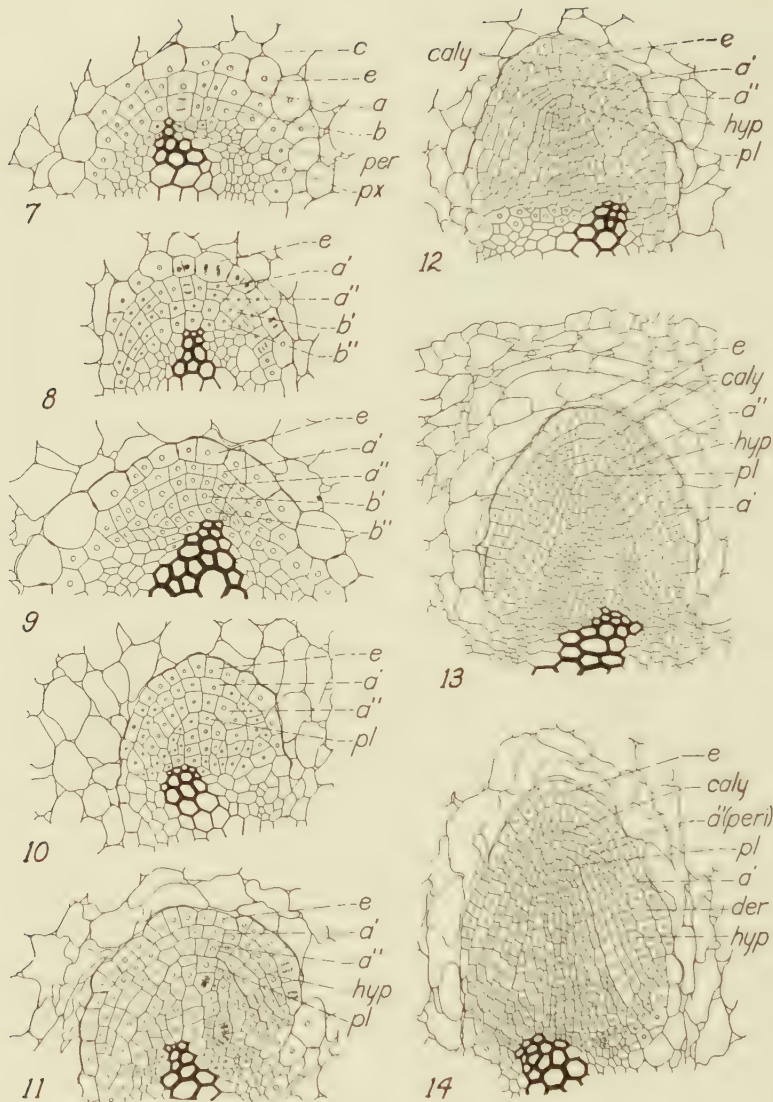
Lateral root histogens

Lateral roots are formed in the ontogeny of the primary axis about the time the thickenings are deposited on the walls of the metaxylem cells. Several lateral roots develop just below the soil level and are usually oriented slightly to one side of the protoxylem point, but

may occur on the same radius directly outside this point (9). Development is initiated by a tangential division of several cells of the pericycle next to a protoxylem point (fig. 7). This division produces two distinct layers (*a*, *b*), each of which in turn again divides by another tangential division (figs. 8, 9). The inner layer (*b*) usually divides somewhat in advance of the outer layer (*a*), as is indicated in figure 8. Figure 9 shows the second tangential division completed, forming four layers of cells, the outer two (*a'*, *a''*) being derivatives of (*a*) and the inner two (*b'*, *b''*) being derivatives of (*b*). A few radial divisions which compensate for the increase in length of overlapping layers occur in the two- and four-layered pericycle, but these divisions usually cause no distortion of the tangential rows. The outer (*a'*) of the four layers derived from the pericycle gives rise to the calyptragen and dermatogen; the next (*a''*) gives rise to the periblem; and the two inner ones (*b'*, *b''*) continue cell activity as a plerome.

By the time four layers of cells have been derived from the pericycle, the endodermis becomes active by radial divisions (9, 17). At this stage the endodermis can easily be identified by the Casparian strips which persist on the radial walls after several subsequent radial divisions in the original cells. The endodermis continues to divide in a plane anticlinal to the developing lateral root, and forms a uniseriate layer of cells about the tip. This layer produced from the endodermis never becomes more than one cell in thickness. Cell activity continues until the lateral root has pushed through the cortex of the primary root into the soil and the cell layer derived from the endodermis is sloughed off with the outer layer of the root cap.

The four layers of cells derived from the pericycle establish the first histogen of the new lateral root. The inner two layers (*b'*, *b''*) continue to divide in all planes and form a conical group of cells, the plerome (fig. 10). Several subsequent divisions follow in the plerome before any tangential divisions take place (periclinal to the new lateral root) in the outer two layers (*a'*, *a''*). All subsequent development and differentiation of the plerome is similar to that described for the primary root. The primary xylem of the lateral root is diarch, and the protoxylem elements lie in the same plane as those of the primary root from which the lateral root originates.



FIGS. 7-14.—Transverse sections of young primary roots showing origin of lateral roots: *px*, primary xylem; *per*, pericycle; *e*, endodermis; *pl*, plerome; *hyp*, hypodermis; *caly*, calyptrogen; *peri*, periblem; *der*, dermatogen; *a*, outer layer of first two layers derived from pericycle; *b*, inner layer of first two layers derived from pericycle; *a'*, outer layer derived from *a*; *a''*, inner layer derived from *a*; *b'*, outer layer derived from *b*; *b''*, inner layer derived from *b*. Derivatives of layer *a'* become the calyptrogen and dermatogen; derivatives of layer *a''* become the periblem; derivatives of layer *b* become the plerome.

Concurrent with increase in the size of the plerome, several radial divisions (anticlinal to the new lateral root) occur in the two outer layers from the pericycle (a' , a''). Following establishment of the plerome, the innermost (a'') of the two outer layers begins periclinal divisions at a point farthest from the tip (fig. 11). The periclinal divisions in this layer (a'') occur successively toward the tip for a time and then their innermost derivatives undergo periclinal divisions. This condition is shown on the right side of figure 11, where the basal part of layer a'' has divided once and the innermost derivative is again undergoing division, as indicated by a mitotic figure. The number of cell layers is increased by successive periclinal divisions of the newly formed innermost layers (fig. 12). Such periclinal divisions are continued progressively toward the tip of the new root, and finally the one to four remaining cells of layer a'' (fig. 13) are divided once periclinally, and the fundamental plan of the periblem (fig. 14) becomes established on the same plan as described for the primary root. The outer layer of the periblem divides only anticlinally, thereby continuing the uniseriate layer which differentiates into the hypodermis. At the tip of the root the innermost layer of the periblem continues to produce, by subsequent divisions, the remaining six to eight layers of the cortex (fig. 14).

By the time the layer a'' and its derivatives have developed to the extent that about three layers of cells are formed about the base of the conical plerome, the outermost layer (a') is divided at the tip by periclinal divisions (fig. 12). This initiates development of a calyp-trogen by forming two layers of cells of which the outer is the root cap while the inner remains meristematic, functioning as a calyp-trogen and later dividing to form the second layer of the root cap. By such subsequent periclinal divisions the root cap is produced and the calyp-trogen is perpetuated as a single layer of meristematic cells overlying the periblem. The laterally oriented cells of the layer a' continue anticlinal divisions as a dermatogen, which is the same as was described for the primary root.

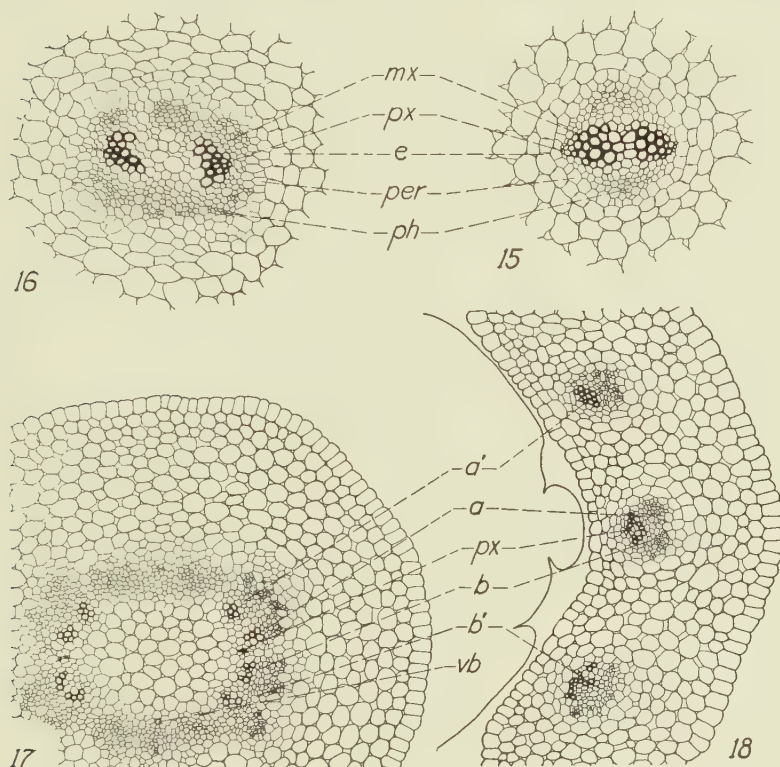
With all the histogens of the lateral root established on the same plan as the primary root, the subsequent growth and differentiation is also as that described for the primary root. There is little accumulation of cell débris or distortion of cortical cells about the tip

of the young lateral root as it grows through the cortex of the primary root. This would seem to indicate that there is some digestion as well as mechanical crushing of the cortical cells as the lateral root develops.

Hypocotyl, cotyledons, and transition

The lower portion of the hypocotyl is rootlike in vascular arrangement. A section made at approximately the ground level shows a primary xylem plate without central pith and two phloem groups alternate with the protoxylem elements (fig. 15). The central portion of the stele of the hypocotyl is parenchymatous at approximately one-fourth of its length above ground. There is a gradual reorientation of the primary vascular tissue at successively higher levels throughout the hypocotyl and basal part of the cotyledons. In the lower one-fourth of the hypocotyl the two phloem groups diverge and form four phloem regions which at a higher level are equally distributed at 90° intervals about a cross-section (fig. 16). Phloem which is associated with the first two leaves of the epicotyl is soon differentiated in the hypocotyl, and anastomoses at the point at which the phloem groups of the lower hypocotyl diverge. In the central region of the hypocotyl the metaxylem elements are differentiated in a tangential position with respect to the protoxylem points. This forms four metaxylem groups, each of which is accompanied by a corresponding phloem strand located outward on the same radius. Somewhat above the middle region of the hypocotyl, the four metaxylem groups with the related phloem groups are each differentiated as two groups, so that eight vascular strands associated with the cotyledons are formed. At successively higher levels throughout the hypocotyl the four metaxylem groups (fig. 3 *a, a', b, b'*) related to each protoxylem point become oriented more and more definitely lateral to the protoxylem (figs. 3, 17, 18). In the upper region of the hypocotyl this orientation of the eight cotyledonary bundles results in two widely separated groups, each of which becomes a cotyledonary trace. At the point of divergence of a cotyledon the two lateral bundles of metaxylem and accompanying phloem (*a', b'*) of each group of four become widely separated from the two middle bundles (*a, b*). Each of these lateral bundles (*a', b'*) is a

bundle of the trace of a lateral vein of the cotyledon. At successively higher levels in the upper hypocotyl and basal part of the cotyledon,



FIGS. 15-18.—Transverse sections of seedling 6 days old showing transition from root to cotyledon. Fig. 15, transection about 1. mm. below ground level. Fig. 16, transection in middle region of hypocotyl showing parenchymatous center of stele and early breakdown of protoxylem. Fig. 17, transection of upper region of hypocotyl showing 8 metaxylem groups with associated phloem (also breakdown of protoxylem). Fig. 18, transection at base of cotyledons and epicotyl showing level where two metaxylem groups anastomose and showing also two phloem groups which retain their identity until a higher level in the cotyledon (two lateral veins of cotyledon also shown): *mx*, metaxylem; *px*, protoxylem; *e*, endodermis; *per*, pericycle; *ph*, phloem; *vb*, vascular bundle of first leaf; *a*, *b*, bundles which anastomose and form double bundle of cotyledon; *a'*, *b'*, lateral metaxylem bundles which form lateral bundles of cotyledon.

the two metaxylem groups (*a*, *b*) nearest the protoxylem become oriented more and more in an endarch relationship to the proto-

xylem. In the base of the cotyledon the two metaxylem groups are differentiated in an endarch relation to the protoxylem, and at this level the two groups of metaxylem anastomose (fig. 18) and form the median double bundle (fig. 26) of the cotyledon (14). The two phloem groups associated with the median bundle retain their identity for 2-3 mm. farther into the cotyledon and then anastomose (fig. 18).

Figure 3 shows the transition in a semidiagrammatic manner. Each strand shown in this diagram may be interpreted as a bundle of associated xylem and phloem. The vascular elements of the epicotyl are differentiated somewhat later than the vascular system related to the cotyledons. Figure 17 shows a zone of phloem which is associated with the first leaves. These bundles are differentiated as endarch, collateral bundles throughout the hypocotyl, where they may anastomose with the lateral arcs of metaxylem and accompanying phloem in the lower hypocotyl, or may end blindly in the parenchyma. The bundles related to the first two leaves are small in the middle and lower hypocotyl, and usually anastomose with the lateral arcs of metaxylem and accompanying phloem; while the other bundles related to the epicotyl end blindly and have vascular connection only through secondary tissue. The transition is actually a root cotyledon transition as there is no reorientation of bundles that have relationship to the epicotyl.

In order to follow the transition in the middle and upper hypocotyl, it is necessary to examine plants before the hypocotyl has greatly elongated. Plants six days old, or when the cotyledons first come above the soil, have well differentiated primary xylem throughout the hypocotyl and cotyledons. As the hypocotyl elongates the protoxylem is crushed and most of it is obliterated by digestion. Later the early metaxylem breaks down and finally nearly all of the primary xylem disappears in the middle and upper hypocotyl. All of the primary xylem elements in the upper region of the hypocotyl have spiral wall thickenings. This is the last region to cease elongation, and the consequent and relatively continuous stretching causes a collapse of the spiral vessels.

By the time plants are 13 days old, most of the primary xylem of the upper region of the hypocotyl is crushed and is partly resorbed (fig. 19). CHAUVEAUD (4) has reported breakdown and complete

resorption of primary xylem in *Mercurialis* and *Allium*, much the same as was observed in *Linum*. In *Linum* there are fragments of the primary xylem that remain after 30 days, and may remain through the life of the plant, without being completely resorbed. Many cross-sections through the upper hypocotyl show no protoxylem and very little metaxylem, while at other levels fragments of

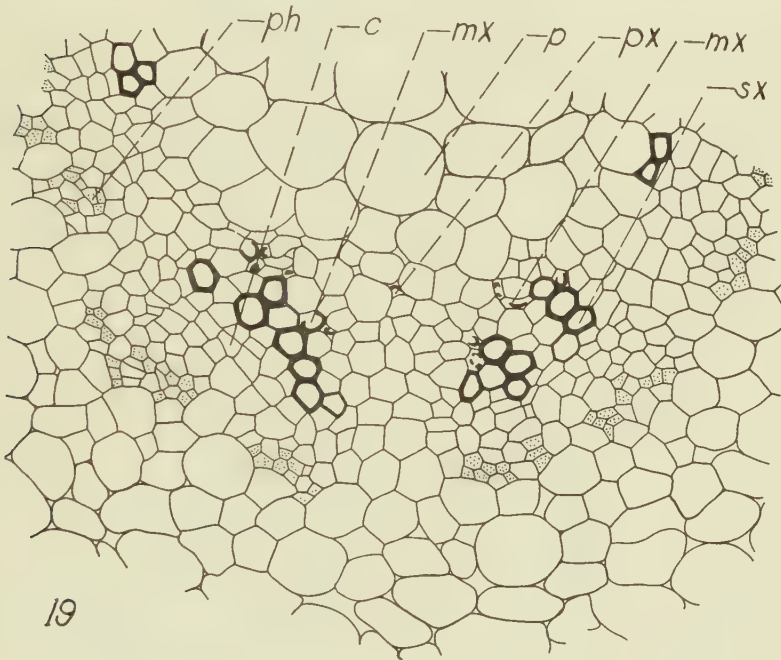


FIG. 19.—Transverse section in upper region of hypocotyl showing early breakdown and resorption of primary xylem: *c*, cambium; *mx*, metaxylem; *px*, protoxylem; *sx*, secondary xylem; *p*, pith; *ph*, phloem.

the elements can be found between the parenchyma cells. The ephemeral nature of the primary xylem probably led to some misinterpretation when GÉRARD (5) and TOGNINI (15) described the transition from root to stem in *Linum*. TOGNINI observed the breakdown of primary xylem but did not distinguish protoxylem and metaxylem in respect to their time and position of differentiation in the upper region of the hypocotyl.

The cotyledons are almost sessile, and in young seedlings they are

somewhat wider than the hypocotyl at their point of divergence. In the young seedlings they are made up of very compact storage cells.

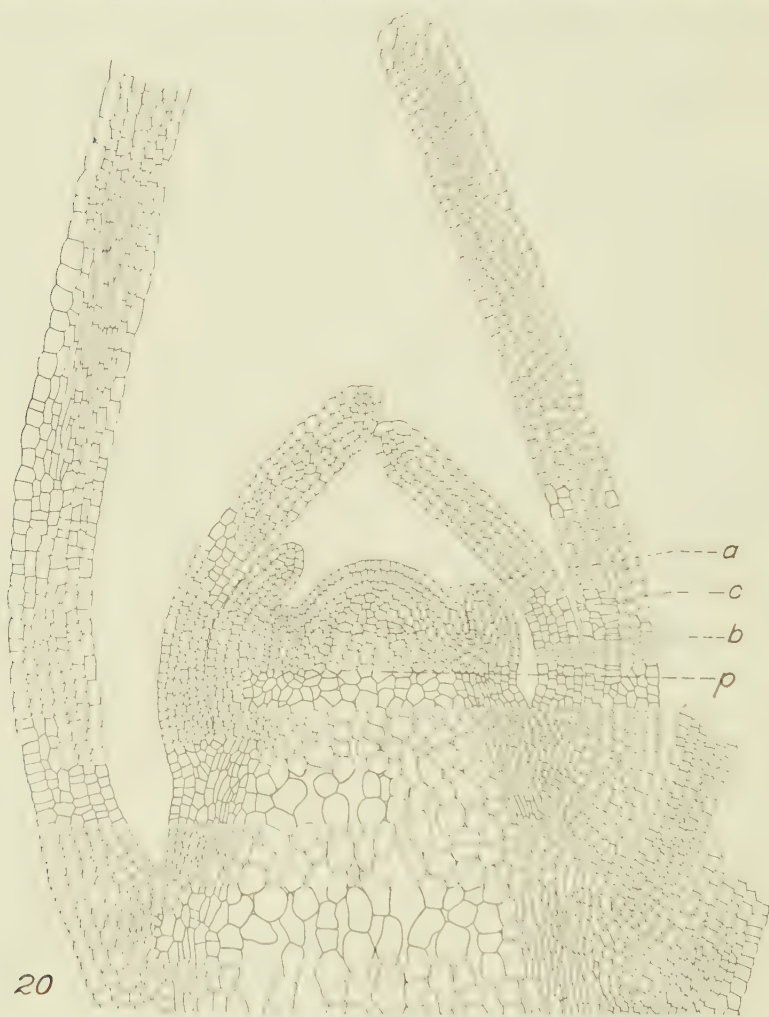


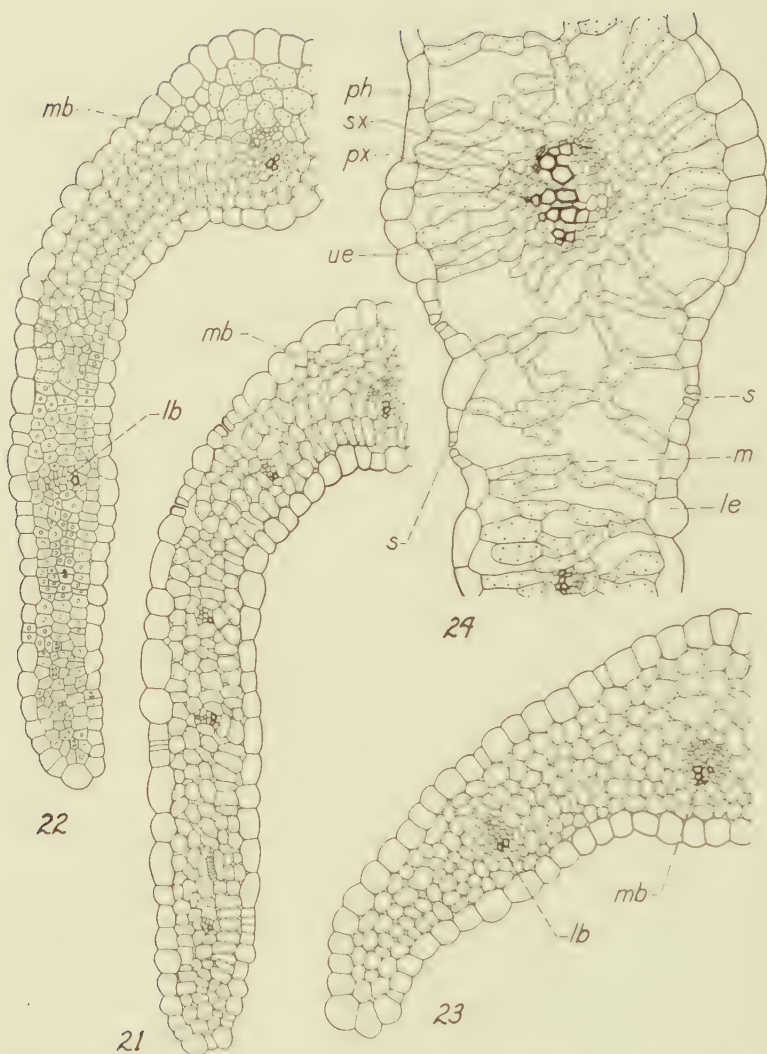
FIG. 20.—Median longitudinal section of growing point showing several stages of ontogeny of leaf: *a*, section of very young primordium; *b*, *c*, older primordia showing early development of provascular strands; *p*, pith.

Two or three of the adaxial layers take the form of a palisade layer, while the lower mesophyll consists of four or five layers of more

rounded, compact cells. Protoxylem elements are differentiated in the median bundle of the cotyledon in seeds placed in conditions favorable for germination for a period of 24 hours, and in some cases may be differentiated in the mature seed. Usually a few scattered phloem cells can be identified in the provascular strands of the young cotyledons before any wall deposits can be noted in the cells of the primary xylem. After the cotyledons are above the ground they continue to thicken and enlarge for a period of about 15 days. The photosynthetic tissue of the mature cotyledon has in general the structure of a mature leaf (fig. 24), except that it is considerably thicker and has smaller air spaces. Both the upper and lower epidermis have a considerable number of stomata with large air spaces underlying them. The closed, netted venation of the cotyledon and leaf is essentially the same (figs. 26, 27). Except in a short region in the median vein, the vascular bundles of the cotyledons are collateral and develop no secondary tissue, while the large leaf bundles develop considerable secondary xylem and phloem. The xylem elements of the cotyledons have spiral or loosely netted wall thickenings. After about 30 days the cotyledons become yellow and die.

Epicotyl and foliage leaves

The young epicotyledonary axis is surrounded by the bases of the cotyledons and develops very slowly for the first eight to ten days. The growing point cuts off leaf primordia which have an irregular phyllotaxy. The leaves at the first three nodes are usually in alternate pairs. Above the fourth node the leaves are arranged spirally, with one leaf at a node in a more or less indefinite phyllotaxy. TOGNINI (15) classified the arrangement of the upper leaves in a $2/5$ system of phyllotaxy but states that frequently there are exceptions, even in different parts of the same plant. In this investigation no definite phyllotaxy could be established for any one plant, except for short intervals which suggested a $2/5$ system. Occasionally three leaves are in a whorl at one of the lower nodes, and often the sixth, seventh, or eighth leaf is bilobed (fig. 25). A bilobed leaf may be broad and cleft almost to the base, or narrow with a very small cleft at the tip. The vascular system of such leaves shows them to have the form of a double leaf or of two leaves nondiverged in varying



FIGS. 21-24.—Figs. 21-23, transverse sections of first leaf when its total length is 2.5 mm. Fig. 21, transection 0.9 mm. from tip of leaf where cell divisions have ceased. Fig. 22, transection 1.8 mm. from tip of same leaf showing region of meristematic activity in lamina. Fig. 23, transection 0.1 mm. from base of same leaf showing level where cell divisions have ceased: *mb*, median bundle; *lb*, lateral bundle.

Fig. 24, Transverse section through median vein of mature leaf from middle region of stem: *px*, primary xylem; *ph*, phloem; *sx*, secondary xylem; *s*, stoma; *le*, lower epidermis; *ue*, upper epidermis; *m*, mesophyll.

degrees. A double leaf usually has two perfect leaf traces. The lateral bundles of the two traces anastomose and form a small bundle which is median in the double leaf (fig. 25).

A leaf primordium arises from tissues of at least the three outermost cell layers of the growing point and forms a conical mass of cells. It elongates and broadens by general meristematic activity until it becomes a somewhat flattened, finger-like projection. The procambial strands are differentiated early (fig. 20 *c*). General meri-



FIGS. 25-27.—Diagrams showing venation of cotyledon and foliage leaves: fig. 25, double leaf; fig. 26, cotyledon; fig. 27, typical early foliage leaf.

stematic activity increases the length of a flattened primordium to about one-sixth or one-fifth the length of a mature leaf. Continued meristematic activity laterally and toward the adaxial surface results in formation of the lamina (fig. 22). During formation of the lamina other cells of the mesophyll undergo enlargement and further differentiation, and the vascular bundles are more completely differentiated. Meristematic activity first ceases in the tip of the leaf, next in the basal portion, and last in the basal portion of the lamina (figs. 21, 22, 23). After the young leaf has attained one-sixth to one-fifth its mature size all further expansion is by enlargement, stretching, and pulling apart of cells.

At maturity the mesophyll is very loose, with large intercellular spaces (fig. 24). There are about four cell layers of somewhat elongated cells which are essentially alike and not differentiated into a definite palisade and spongy parenchyma. In the mesophyll there is a complicated network of veins of various sizes. The median vein and sometimes the two lateral veins develop considerable secondary tissue. The smaller veins have fewer and fewer elements, and either form a closed netted system or may end blindly in the mesophyll. The epidermal cells are irregular in size and have a thin deposit of cutin on the outer walls. The stomata are numerous and occur in about equal numbers on both sides of the leaf. The guard cells are subtended by accessory cells.

The fundamental plan of the primary vascular system of the stem is differentiated early, in the form of procambial strands in the leaf primordia and growing point. The leaf primordia are close together, so that a length of 0.1–0.15 mm. of the growing point includes 10–20 nodes and internodes. The procambial strands are differentiated in the leaf primordia and to a distance of 0.1–0.15 mm. in the meristematic zone of the growing point at essentially the same time (fig. 20 *b*). Even in this short distance the strands extend through 10–20 nodes, and as the internodes elongate the vascular system is elongated with the same fundamental plan and with no differentiation of anastomosing bundles. The mature primary vascular system is then made up of foliar bundles, all of which are collateral. The leaf trace consists of three large bundles which differentiate as separate bundles in the cortex and anastomose at the point at which the trace becomes a part of the vascular ring in the axis. The bundles are largest where they diverge from the stele, and are gradually smaller at lower levels, where they end blindly after extending through 15–20 nodes. The vascular connections within the stele are by secondary tissues and not by anastomosing of bundles. Usually 8–10 foliar bundles are differentiated below the cotyledonary node. Bundles associated with the first pair of leaves anastomose with the lateral arches of the metaxylem of the rootlike lower hypocotyl; but even in this case the bundles are small at the lower level and vascular connection is principally by secondary tissues. Other foliar bundles below the cotyledonary node end

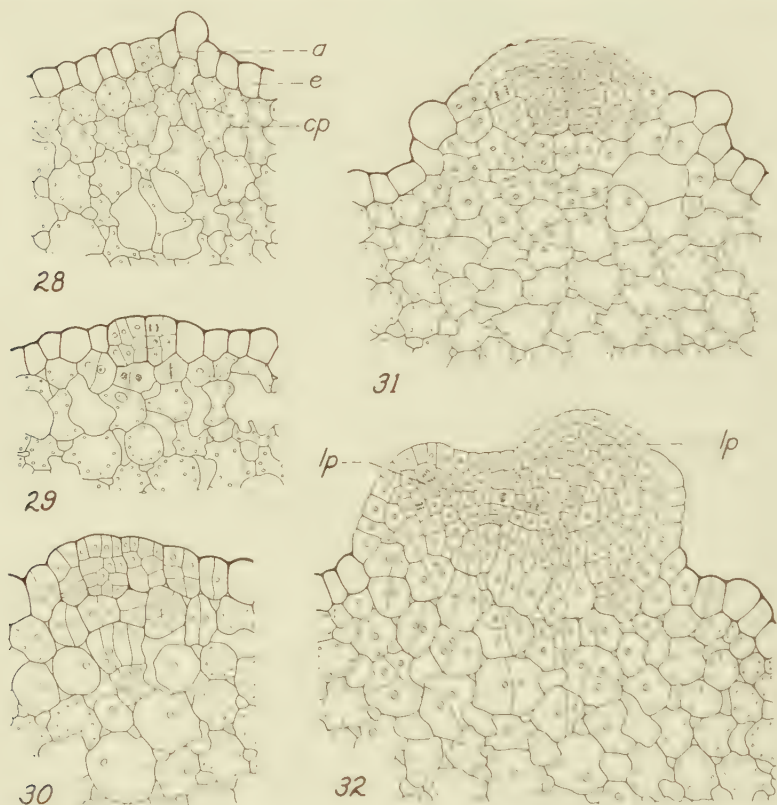
blindly in the parenchyma between other vascular bundles. As the primary tissues mature, fascicular and interfascicular cambium is differentiated. The tissues formed by the activity of fascicular and interfascicular cambium form a continuous vascular cylinder of secondary tissue, which establishes the only vascular connection between the primary vascular elements.

Adventitious buds from hypocotyl

Young plants readily produced shoots when the growing point was injured or removed. When the injury was above the cotyledon an axillary bud became active and produced a shoot. All organized buds were removed by severing seedlings of various ages at different levels below the cotyledons. All plants not more than 10 days old produced 5–20 buds on the remaining lower part of the hypocotyl, even when the top had been removed to a few millimeters above the ground level. When the tops of plants 60 days old were severed below the cotyledons, approximately 60 per cent of the plants died without forming buds; while plants less than 10 days old produced small buds along the remaining hypocotyl within 6–8 days after severing. These buds do not all start development at the same time. A microscopic examination shows that while buds on one part of the hypocotyl are large enough to show small leaves, many other buds are in earlier stages of development. After 8–10 buds begin to show small leaves, one bud usually outgrows all the others. This one bud may be at any level in relation to the others, and is not necessarily the first one to have made its appearance on the hypocotyl. In a few instances more than one bud may continue development. The shoot that develops from such an adventitious bud continues as an unbranched axis until the formation of a flower cluster, which matures at approximately the same time as those of plants of the same age left uncut. At maturity the adventitious shoot has attained about one-half the height of a plant left uncut. Many of the cells at the cut surface of the hypocotyl collapse and die, and little or no wound parenchyma is formed by the underlying cells.

After a plant has been severed, the cortical region of the remaining part of the hypocotyl develops large intercellular spaces which give it an appearance similar to the mesophyll of a leaf (figs. 28, 29).

The bud is initiated by division of an epidermal cell. This first division is quickly followed by a second, which gives a four-celled stage such as is shown by figure 28. Epidermal cells adjoining the derivatives of the first meristematic cell in turn become active, so that



FIGS. 28-32.—Transverse sections of hypocotyl showing development of adventitious bud after plant has been severed below cotyledons: *a*, four derivatives of single epidermal cell; *e*, epidermis of hypocotyl; *cp*, cortical parenchyma of hypocotyl; *lp*, leaf primordia of adventitious bud.

as many as five or six epidermal cells become meristematic (figs. 29, 30). The identity of the derivatives of an original epidermal cell can be determined by a heavy cell wall about the group, even after four or five subsequent divisions (fig. 30).

When one or two epidermal cells have become active, the under-

lying, adjoining cortical cells lose their chloroplasts, become less vacuolate, and begin cell divisions (fig. 29). These cells continue to divide and enlarge, so that the large intercellular spaces are filled and other adjoining cortical cells in turn become active. The activity of the cortical parenchyma continues until an active zone of cells extends to the endodermis. Growth of the cortical parenchyma results in elimination of the intercellular spaces. These new parenchymatous cells differentiate in place and do not contribute to the elongation of the new axis.

Derivatives of the original epidermal cells by subsequent divisions eventually constitute the axis of the adventitious bud. A dome-shaped group of cells is first formed (fig. 31), and then by rapid cell growth two or three leaf primordia are developed about the periphery of the dome-shaped group of meristematic cells. The first leaf primordia develop rapidly, and the flat group of cells between them later becomes a dome-shaped growing point which is similar to the epicotyl (figs. 32, 33). Vascular bundles are differentiated in the first two or three leaves of the new bud by the time the growing point is well established, and before any vascular system has been established between the stele of the hypocotyl and the new bud. Protoxylem elements are differentiated in the first leaves of the new bud before cell division has begun in the inner layers of the cortex of the hypocotyl (fig. 33). After the cells of the inner cortex have become meristematic, vascular tissue is differentiated progressively inward from the new bud to the endodermis. After many tracheids have been differentiated from the derivatives of the cortical parenchyma, a number of simultaneous tangential and radial divisions take place in the endodermis (fig. 34). Some of the derivatives of the endodermal cells soon differentiate into tracheids, and establish continuity with the tracheids which have differentiated from cells in the pericycle and in the phloem parenchyma (fig. 35). Figure 35 indicates the relative size of the adventitious shoot before vascular connection is established with the vascular elements of the primary axis of the hypocotyl. Very few cells other than elongated parenchymatous cells can be identified as phloem. By the time vascular connection is established with the vascular elements of the hypocotyl, a layer of cells surrounding the tracheids becomes an active cambium.



FIGS. 33, 34.—Longitudinal sections of hypocotyl showing development and differentiation of adventitious buds after plant has been severed below cotyledons: *e*, endodermis.

In a few weeks the secondary tissues resulting from the multiplication and divisions of the cambium of the hypocotyl and those from the cambium of the newly formed shoot develop a plant axis which is almost straight.



FIG. 35.—Longitudinal section through hypocotyl and an adventitious bud showing differentiation of vascular connection between the new bud and stele of hypocotyl.

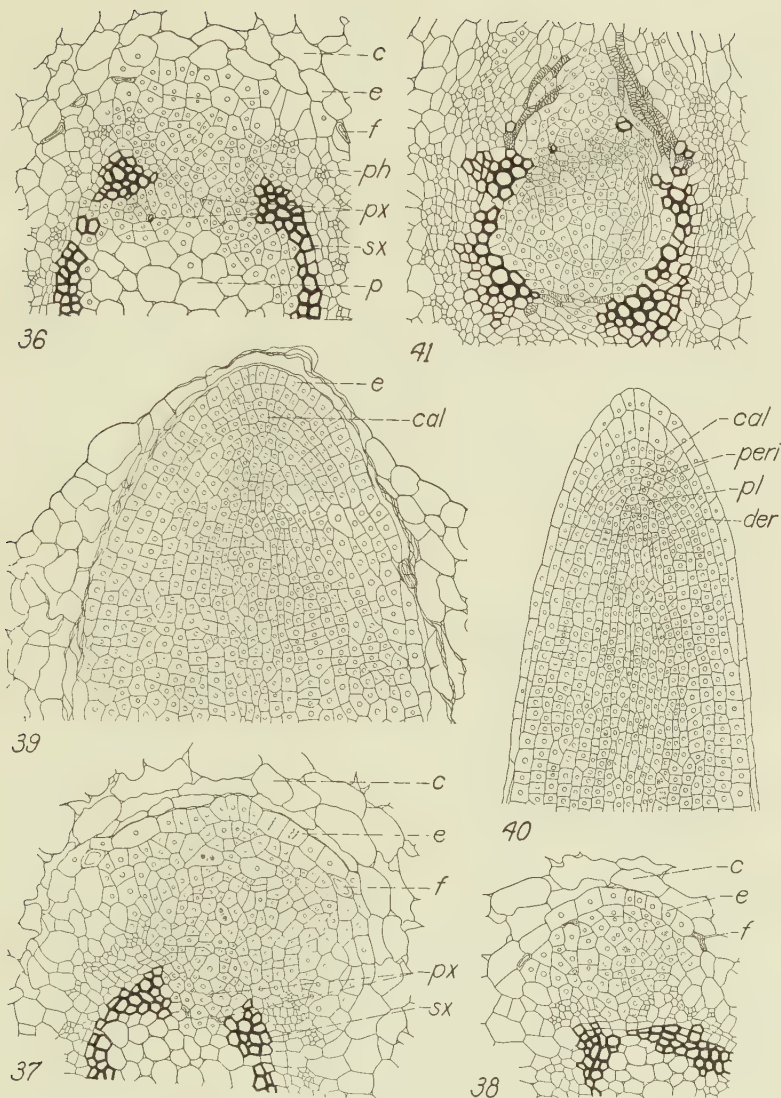
Adventitious roots from hypocotyl

Plants six to ten days old were severed at the middle region of the hypocotyl, and the upper part of the plant set with the hypocotyl in moist soil so that the cotyledons were just above the soil level. Elongation of the hypocotyl soon lifted the cotyledons from 1 to 3 cm. above the ground level, depending on the age of the

plant at the time of cutting. All the cuttings of young plants produced two to five roots along the lower part of the hypocotyl. Roots developed even when the remaining hypocotyl was only long enough to hold the cotyledons in contact. Cuttings of the epicotyl made several nodes above the cotyledons also produced roots.

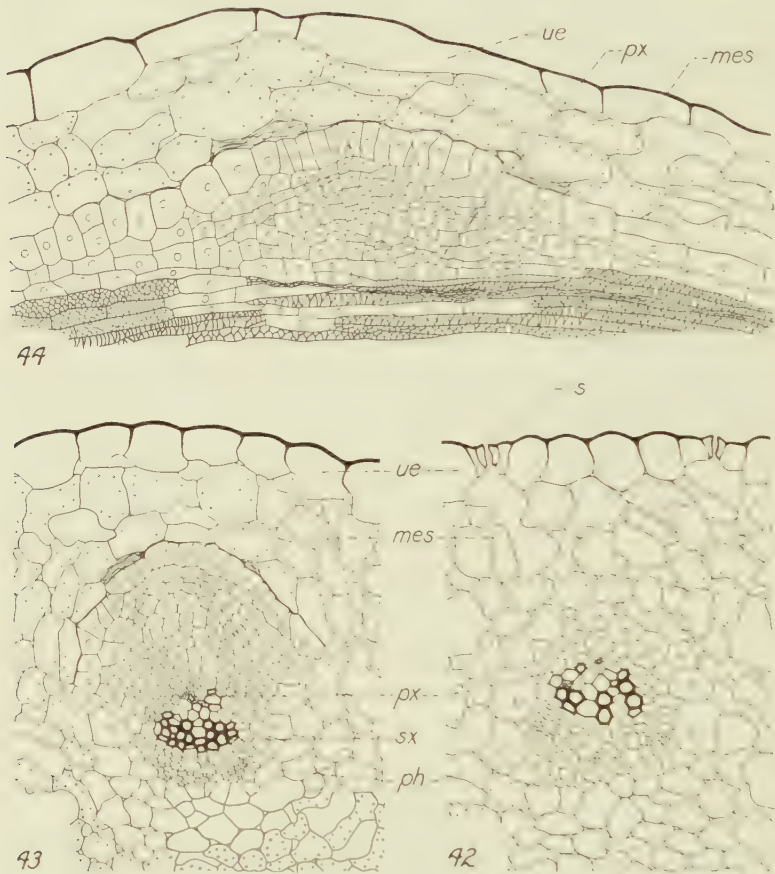
In the hypocotyl a V-shaped ray of parenchymatous tissue extends outward from each protoxylem strand, which is continuous with the protoxylem of the median bundle of a cotyledon, and the adventitious roots are usually initiated by a general activity of the cells in these parenchymatous rays. These roots then come from the hypocotyl in two rows, each of which is directly below the midvein of one of the cotyledons. Often the general activity extends from the outermost layer of the pericycle to and including the parenchymatous cells about the primary xylem (fig. 36). Almost all of the primary xylem is broken down and resorbed so that cell activity involves even the pith. Cell divisions continue in all planes, thus forming a dome-shaped group of cells which pushes outward into the cortex. The endodermis of the hypocotyl can be identified by the early development of pericyclic fibers (1). The endodermis becomes active by cell divisions in a radial plane (fig. 37). This uniseriate layer of endodermal cells keeps pace with the subsequent growth of underlying cells, and forms a layer over the root cap which does not break down until the new root grows 1-2 mm. into the soil. The new root grows by general activity of the cells, with no organized histogens until about the time it pushes through the cortex into the soil. Then at the tip of the new root the cell layer underneath the endodermal layer begins to divide periclinally, thus becoming a true calyptragen which continues to function as the calyptragen described for the primary and lateral roots (fig. 39). The periblem and plerome are not definitely established until the root has grown 2-3 mm. into the soil. Figure 40 shows an adventitious root with total length of 2.5 mm., in which the periblem and plerome have become established and are functioning as the histogens described for the primary root.

Cell divisions often occur throughout the pith region of the hypocotyl, and form a solid cylinder of parenchymatous cells which remain active for a month or more after the new roots develop (fig. 41). When two roots develop at the same level on the hypocotyl, the cell



FIGS. 36-41.—Transverse sections of cuttings of hypocotyl showing development of adventitious root. Figs. 36, 37, 39, initiation of roots in ray parenchyma in same radius as protoxylem. Fig. 38, initiation of root in pericycle and phloem parenchyma not in same radius as protoxylem. Fig. 40, median longitudinal section of adventitious root with histogens established (root length 2.5 mm. from hypocotyl). Fig. 41, transverse section of hypocotyl, median through an adventitious root, showing cell activity throughout pith region: *c*, cortex; *e*, endodermis; *f*, pericyclic fiber; *ph*, phloem; *px*, primary xylem; *sx*, secondary xylem; *p*, pith; *cal*, calyptrogen; *peri*, periblem; *pl*, plerome; *der*, dermatogen.

activity of the pith is usually more extensive. The cells of this region of the pith may become differentiated as wound tracheids or remain as relatively thin-walled cells.



FIGS. 42-44.—Figs. 42, 43, transverse sections of cotyledon made at right angles to median vein showing development of adventitious root: *ue*, upper epidermis; *mes*, mesophyll; *px*, primary xylem; *sx*, secondary xylem; *ph*, phloem; *s*, stoma. Fig. 42, first activity of parenchyma surrounding median vein. Fig. 43, early development of adventitious root on adaxial side of median vein of cotyledon. Fig. 44, longitudinal section through median vein of cotyledon showing origin of adventitious root on adaxial side of bundle.

Some adventitious roots of the hypocotyl are initiated by a general cell activity in the pericycle and phloem parenchyma (fig. 38).

In this case they are not in the same plane as the median bundles of the cotyledons. The subsequent development is the same as that described for the roots arising in the plane of the median bundles of the cotyledons.

At the cut surface of the hypocotyl some groups of parenchymatous cells of the cortex, pericycle, phloem, and sometimes regions of the pith divide several times without the derivative cells enlarging. Except in these regions of parenchyma, no extensive wound tissue is formed.

BEALS (3), in describing regeneration phenomena in flax, states: "First the epidermis divides and then the innermost row of those cells and the stimulated cells of the region just beneath form the regenerated part, root or shoot (pl. 16)." In the present investigation the shoots were initiated by the epidermis and the roots from tissues of the stele. Cuttings grown on filter paper in sterile dishes, in the manner described by BEALS, developed roots, all of which originated from tissues of the stele in the manner already described.

Adventitious roots from cotyledons

The cotyledons were severed from seedlings and placed on the surface of moist soil. Some were placed horizontally with the adaxial surface up, others with the adaxial surface down, and others vertically with the cut end set about 5 mm. into the soil. About 90 per cent of the cotyledons taken from plants less than ten days old developed three to six roots, while few of the cotyledons from plants twenty days old produced roots when placed on moist soil. The cotyledons developed an extensive root system and grew to be larger and thicker than those not removed from the axis. Often one to six layers of parenchymatous cells at the cut surface of the cotyledon divided several times without the derivative cells enlarging. Sometimes some of the wound tissue near the larger bundles differentiated tracheids. Cotyledons never produced buds but often lived for more than sixty days, while those on the plant usually live as active photosynthetic leaves for about thirty days.

Usually one root came from near each of the three larger veins of the cotyledon, although as many as three roots frequently developed near one vein. The roots pushed through the adaxial surface

of the cotyledon within 3-4 mm. of the cut end, or emerged from the cut surface. When a cotyledon was placed with the adaxial surface up, the roots either emerged from the cut surface or grew up through the epidermis and then curved over the cut end into the soil.

These roots were initiated by the activity of a layer of parenchymatous cells which surrounds the vascular elements of the bundle (fig. 42). In many cases this layer became active about the entire bundle, and in some cases the phloem and xylem parenchyma also became active. In other cases the activity was limited to the adaxial side next to the xylem. The cell activity usually extended 1 mm. or more along the bundle, and the cambium of the larger veins produced considerable secondary tissue (figs. 43, 44). Increased cell activity on the adaxial side of the vein produced a dome-shaped group of cells (figs. 43, 44) which was either pushed through the upper epidermis or grew about parallel to the bundle. When the roots were 2 mm. or more in length, definite histogens were organized similar to those described for the adventitious root from the hypocotyl.

In two cases the root was organized from wound tissue and grew directly from the cut surface of the cotyledon. All other roots that emerged from the cut surface, or pushed through the epidermis, had their origin in the parenchymatous cells on the adaxial side of a vein at a point 1-4 mm. from the cut surface. From the point of origin the roots either grew through the mesophyll parallel to the vein and emerged through the cut surface or pushed through the epidermis on the adaxial surface of the cotyledon. In a few cases the roots originated almost lateral to a vein but were always slightly nearer the adaxial surface.

Summary

1. Germination of the seed and development of the seedling of *Linum usitatissimum* are described.
2. The primary root is diarch. The growing point has clearly defined histogens. The plerome, periblem, and calyptragen give rise to the stele, cortex, and root cap respectively. Laterally to the calyptragen, the dermatogen produces the epidermis by anticlinal divisions.
3. In the development of the vascular elements of the root, two

primary phloem ducts are first differentiated by elongation and breakdown of a single row of cells lying next to the pericycle and alternate with the protoxylem points which are differentiated later.

4. Lateral roots are initiated by regular divisions of some of the cells of the pericycle. One tangential division of these cells of the pericycle forms two layers which in turn divide simultaneously and form four layers of cells. The outer layer later differentiates as the calyptragen and dermatogen, the next as the periblem, and the innermost two as the plerome.

5. In formation of lateral roots the endodermis becomes active and forms a single layer of meristematic cells overlying the root cap.

6. A simple type of transition from root to cotyledons occurs in the hypocotyl and base of the cotyledons. The primary vascular system of the root, hypocotyl, and cotyledons forms a system largely independent of the subsequent epicotyledonary development. The principal vascular connection of the epicotyl and hypocotyl is by secondary tissues.

7. In the upper region of the hypocotyl most of the primary xylem is broken down by elongation, and is for the most part re-sorbed by intervening parenchymatous cells.

8. Development of the epicotyl and foliage leaves is described. All vascular bundles of the epicotyl are collateral foliar bundles which do not anastomose but end in the parenchyma between other vascular bundles. The only vascular connection between the primary vascular bundles of the stele is by a continuous vascular cylinder of secondary tissues which is formed by the activity of fascicular and interfascicular cambium.

9. The basal part of seedlings severed at the middle region of the hypocotyl regenerated five to twenty buds, of which usually only one continued development and became a shoot. Regenerated buds originated by successive division of epidermal cells. Vascular connection between the primary stele of the hypocotyl and the new shoot was initiated by renewed cell activity of cortical parenchyma, endodermis, pericycle, phloem parenchyma, and cambium, in the order named. Tracheids of the vascular system were first differentiated in the leaves of the new bud, and then differentiation occurred progressively inward to the stele of the hypocotyl until con-

tinuity was established between the tissues of the new shoot and the stele of the hypocotyl.

10. Seedlings severed in the middle region of the hypocotyl and the upper parts used as cuttings set in moist soil or placed in moist petri dishes produced adventitious roots from the hypocotyl. These roots originated from parenchymatous tissue which in the same root often involved pericycle, phloem, and pith. The endodermis of the hypocotyl became active and produced a single layer of cells overlying the tip of the developing root.

11. Cotyledons cut from seedlings and placed on moist soil produced adventitious roots and lived for more than two months. These roots originated from a layer of parenchymatous cells on the adaxial side of the larger veins. In no case did these cotyledons produce shoots.

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